114127

# SAMPLING PLAN

KEYSTONE SANITIATION LANDFILL SITE
UNION TOWNSHIP, ADAMS COUNTY, PA

TDD No. 9605-23
EPA CONTRACT No. 68-S5-3002

## 1.0 INTRODUCTION

On 10 May 1996, the U.S. Environmental Protection Agency (EPA) Region III Remedial Project Manager (RPM) Christopher Corbett directed the Roy F. Weston, Inc. (WESTON.), Site Assessment Technical Assistance (SATA) team to provide fish tissue samples and a sampling plan for the Keystone Sanitation Landfill National Priorities List (NPL) Site (Site), in Union Township, Adams County, Pennsylvania.

## 2.0 SITE DESCRIPTION

### 2.1 Location

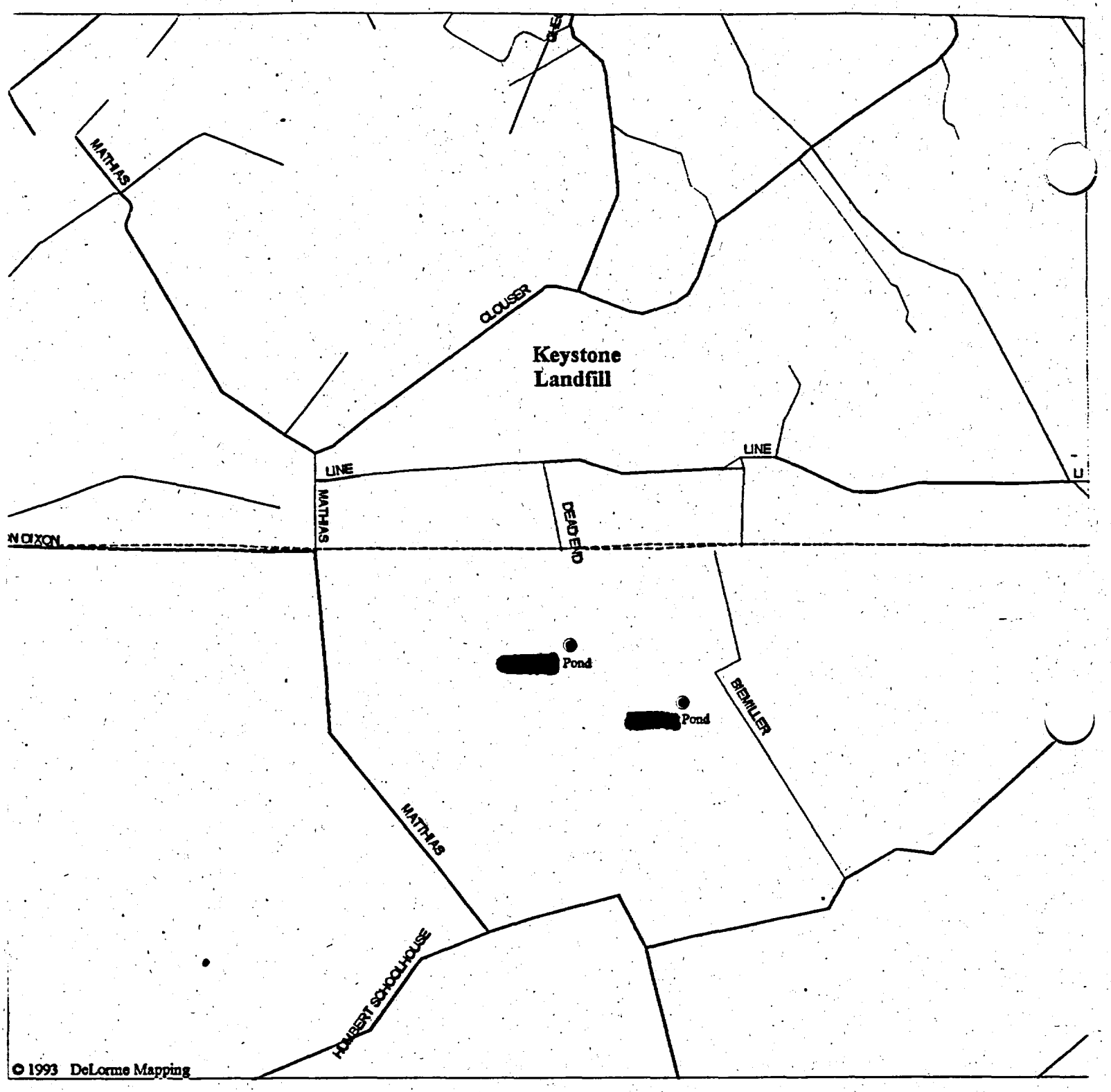
The Keystone Sanitation Landfill NPL Site is located on 40 acres of rural land in Union Township, Adams County, Pennsylvania. The site is approximately 800 ft. north of the Pennsylvania-Maryland border, east of State Route 97. Fish tissue from the [redacted] and [redacted] Ponds will be sampled. The ponds are located south of the site in Carroll County, Maryland (see Figure 1: Site Location Map).

### 2.2 Background

The Site is an inactive landfill owned by the Keystone Sanitation Company. The landfill operated from 1966 to 1990 and was permitted by the Pennsylvania Department of Environmental Protection (PADEP) to receive household and municipal wastes, and certain types of industrial and construction debris. The landfill was constructed without a liner or leachate collection system. A treatment system was installed (SATA, 1995) during a Remedial Action.

The Keystone Sanitation Landfill Site was placed on NPL in July 1987. EPA issued a Record of Decision (ROD) on 30 September 1990 (SATA, 1995). ROD established the Site remedial design that is to be completed in two phases, Operable Unit #1 (OU1)and Operable Unit #2 (OU2). OU1 included the capping of the old landfill area, and the installation of a water treatment system. Currently, OU1 is 60% complete. OU2 called for an off-site contaminant migration investigation of the groundwater.

Thirty-six residents are located within a one mile radius of the site. Approximately 700 residents are located within a five mile radius of the site.




LEGEND

- County Boundary
- Street, Road
- Major Street/Road
- River

Scale 1:15,625 (at center)

1000 Feet

500 Meters

Mag 15.00
Wed May 15 09:40:49 1996

N

**Figure 1:**

**Site Location Map**

AR324249

SATA sampled groundwater for thallium in February and June 1994, and in January and October 1995 (SATA, 1995).

Continuing OU2, the EPA Region III Alternative Remedial Contracts Strategy (ARCS) contractor, Halliburton NUS Corporation, sampled monitoring and residential wells in January and in the Fall 1995. Lead concentrations were detected in three of the residential wells sampled.

## 3.0 PROJECT DESCRIPTION

### 3.1 Objective

The objective of this sampling event is to collect fish tissue samples from two ponds located south of the site. Elevated levels of mercury have been detected in previous sampling events in the area of the landfill and the ponds. Because mercury accumulates in fish tissue, samples will be analyzed for mercury.

### 3.2 Scope of Work

SATA team members will collect four composite samples of fish from each pond. Each sample will consist of five fish from the same species. A whole fish sample and a fish fillet sample will be collected from the top and bottom of each pond. Sampling locations are listed in Table 1.

The following table identifies the proposed sampling locations and their matrices.

Table 1
Sample Locations

| Sample Number | Location | Matrix |
|---|---|---|
| BrP1(W) | [redacted] Pond Top | Fish Tissue |
| BrP2(F) | [redacted] Pond Top | Fish Tissue |
| BrP3(W) | [redacted] Pond Bottom | Fish Tissue |
| BrP4(F) | [redacted] Pond Bottom | Fish Tissue |
| RuP1(W) | [redacted] Pond Top | Fish Tissue |
| RuP2(F) | [redacted] Pond Top | Fish Tissue |
| RuP3(W) | [redacted] Pond Bottom | Fish Tissue |
| RuP4(F) | [redacted] Pond Bottom | Fish Tissue |

### 3.3 Data Use

Analytical results will be incorporated into an ecological risk assessment.

## 4.0 SAMPLING PROCEDURES

### 4.1 Sample Collection

All sampling activities will be performed between 4 and 5 June 1996 . EPA approved or SATA Standard Operating Procedures (SOPs) will be utilized whenever possible. Sample collection activities are summarized below.

#### 4.1.1 Fish Tissue Matrix

Four composite fish samples will be collected from two ponds. The field and laboratory methods will follow the guidelines outlined in the following:

- A Compendium of Superfund Field Operations Methods (Attachment 1).
- Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters (Attachment 2).

Fish samples will be collected by seining the pond. Electroshock will be employed in the event that seining is not feasible. Seining may not be successful if the pond is too deep or the bottom of the pond has a rough configuration. Collected fish will be identified by class in the field and measured for total weight and length. Two composite samples from the game/predator species (i.e., largemouth bass, pumpkinseed, or bluegill) and two composite samples from the bottom feeder species (i.e., bullhead, catfish, or carp) will be collected from each pond. The two composite samples from each group will consist of one whole fish sample and one fillet sample. Depending on field conditions, the whole fish and fillet fish composite samples will consist of five fish from the same species. Each sample will contain at least 200 grams of fish tissue and the smallest fish in the sample will not be less than 75% the length of the largest fish.

Fish will be individually wrapped in aluminum foil with the dull side in contact with the fish and be placed in watertight plastic bags with the rest of the composite sample. The fish will be stored in a cooler with dry ice for shipment to the laboratory. Sample handling prior to shipment will be minimized and fin spikes will be sheared to minimize puncturing of the sample bags. Sample labels will include the site name, the date, the collector's name, the sample location, the sample identifier, the fish species, the sampling technique, the sample type (whole or fillet), sex and age (if possible), and comments.

Prior to analysis, each composite sample, both whole and fillet, will be homogenized at the laboratory by a tissue grinder called a Bassimatic.

4.2 Sample and Equipment Decontamination

Prior to sampling, the fillet board and table will be rinsed with the local water supply. Knives will be cleaned with acetone and the table and fillet board will be wiped with an acetone rinse. The knives, table and board will then be rinsed with the local water source or distilled water. The table and knives will be rinsed with distilled water between specimens.

## 5.0 ANALYTICAL PARAMETERS

Table 2 below provides a summary of the matrices to be collected, parameters to be analyzed, analysis methods, sample containers needed, and detection limits required for this sampling event.

Table 2
Summary of Analytical Parameters

| Sample Location | Matrix | Analytical Parameter | Test Method | Preservatives Used | Special Detection Limits Needed |
|---|---|---|---|---|---|
| BrP1(W) | Fish Tissue | Mercury | CLP Protocol* | Freeze (-18°C) | 0.05 µg/g (dry weight) |
| BrP2(F) | Fish Tissue | Mercury | CLP Protocol* | Freeze (-18°C) | 0.05 µg/g (dry weight) |
| BrP3(W) | Fish Tissue | Mercury | CLP Protocol* | Freeze (-18°C) | 0.05 µg/g (dry weight) |
| BrP4(F) | Fish Tissue | Mercury | CLP Protocol* | Freeze (-18°C) | 0.05 µg/g (dry weight) |
| RuP1(W) | Fish Tissue | Mercury | CLP Protocol* | Freeze (-18°C) | 0.05 µg/g (dry weight) |
| RuP2(F) | Fish Tissue | Mercury | CLP Protocol* | Freeze (-18°C) | 0.05 µg/g (dry weight) |
| RuP3(W) | Fish Tissue | Mercury | CLP Protocol* | Freeze (-18°C) | 0.05 µg/g (dry weight) |
| RuP4(F) | Fish Tissue | Mercury | CLP Protocol* | Freeze (-18°C) | 0.05 µg/g (dry weight) |

RB Rinsate Blank
W Whole
F Fillet
* Specific test method is to be determined.

## 6.0 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) PROCEDURES

This sampling plan is designed to satisfy the Office of Emergency and Remedial Response Data Quality Objectives for Superfund, EPA/540/R-93/078, PB94/963204, September 1993.

6.1 Quality Control of Field Activities

This sampling plan is designed to satisfy the Office of Emergency and Remedial Response Data Quality Objectives Process for Superfund, EPA/540/R-93/078,PB94/963204, September 1993. The SATA Site Leader will be responsible for ensuring that sample quality and integrity are maintained in accordance with the SATA Quality Assurance Project Plan, and that sampling labeling and documentation procedures are performed as described in Section 6.3 of this sampling plan.

6.2 Sample Packaging and Storage

Each fish of the composite sample will be wrapped in heavy-duty aluminum foil. Each fish will be labeled and fish from the same composite will be placed in labelled watertight plastic bags. Each bag will be placed in a cooler of dry ice to preserve the samples and sample documents will be affixed to the underside of each transport container lid. The lid will be sealed with shipping tape and custody seals will be affixed to the transport container. Transport containers will be labeled with the origin and destination locations.

Regulations for packaging, marking, labeling, and shipping of hazardous materials and wastes are promulgated by the U.S. Department of Transportation (DOT). Air carriers which transport hazardous materials, in particular, Federal Express, require compliance with the current International Air Transport Association (IATA) Regulations, which applies to shipment and transport of hazardous materials by air carrier. SATA will follow IATA regulations to ensure compliance.

6.3 Field QC

Field QC will consist of rinsate blanks and sample documentation as referenced in SATA SOP No. 103, Chain of Custody Documentation and SATA SOP No.101, Logbook Documentation. Field quality control (QC) for this sampling event will be provided by a rinsate blanks. Rinsate blanks will be taken to test for effectiveness of decontamination procedures.

6.4 Laboratory QC

Laboratory QC will consist of all QC stated in the Contract Laboratory Program (CLP) Statement of Work (SOW) and include all forms and deliverables required by the SOW.

### 6.5 Data Validation

Data validation will be performed by EPA Region III Central Regional Laboratory's Quality Assurance Members in accordance with EPA Region III Modifications to the EPA Contract Laboratory Program (CLP) National Functional Guidelines for Data Review.

## 7.0 REPORTS

Information gathered from this sampling event will be compiled into a Trip Report. The report will include the data collection methods, sample locations, data summary tables with qualifiers applied during data validation, and a Data Quality Report. The trip report will be submitted to EPA upon completion.

## 8.0 LIST OF DELIVERABLES

The following list of deliverables includes the dates that each will be completed. The sampling plan/work plan will be completed as soon as possible. The trip report and data quality report will be completed 15 days after receipt of the analytical data. Analytical results can be expected approximately a month after the laboratory receives the samples. Field collection has been scheduled and the samples will be shipped the day following the sampling event.

Table 3
Deliverables and Due Dates

| Deliverable | Due Dates |
|---|---|
| Sampling Plan | 20 May 1996 |
| Field Collection | 4-5 June 1996 |
| Trip Report | 22 July 1996 |
| Data Quality Report | 22 July 1996 |

## 9.0 REFERENCES

EPA (U.S. Environmental Protection Agency). 1987. *Compendium of Superfund Field Operations Methods*. Office of Emergency and Remedial Response, OSWER Directive 9355.0-14. EPA/540/P-87/001, December.

Klemm, D.J., Q.J. Stober, and J.M. Lazorchak. 1993. *Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters*. Environmental Monitoring Systems Laboratory. EPA/600/R-92/111, March.

SATA (Site Assessment Technical Assistance). 1995. Sampling Plan for Keystone Sanitation Landfill Site from the October 1995 Sampling Event, , Delran, NJ.

Attachment 1

A Compendium of Superfund
Field Operations Methods

EPA/540/P-87/001
(OSWER Directive 9355.0-14)
December 1987

# A Compendium of Superfund Field Operations Methods

Office of Emergency and Remedial Response
Office of Waste Programs Enforcement
U.S. Environmental Protection Agency
Washington, DC 20460

titative data relative to benthic colonization in areas where substrate conditions may not allow invertebrate colonization or where organisms are scarce, making other collection efforts difficult.

The most common standard samplers are the multiplate or Hester-Dendy sampler and the basket sampler (Exhibit 12A-5). The multiplate sampler is positioned (preferably) in the top meter of water, using floats and stainless steel cable, for 4 to 6 weeks. For maximum retention of organisms during retrieval, the sampler is placed in a bag or large dip net while still positioned in the water.

A basket sampler is a cylindrical basket containing approximately 30 rocks of equal size. This device is often used in creeks and rivers where rocks are the preferred habitat for most invertebrates. Such samplers are also left in place for 4 to 6 weeks.

Organisms removed from either artificial substrate are processed using techniques described in Subsection E3.

## C6. In Situ Bioassays

During this procedure, local invertebrates from a comparatively clean area or invertebrates raised in laboratories under known conditions are confined in traps and held at the site and at a reference site to determine the acute toxicity of the area of suspected contaminants or to determine whether bioaccumulation is occurring.

Approximately 40 to 50 organisms such as bivalves or crayfish are obtained. Then 10 to 15 organisms are placed in two cages—one for the test area and one for the reference area. If the purpose of the study is to determine bioaccumulation, 10 to 15 additional specimens are sacrificed and processed immediately to establish baseline conditions. The test and reference cages are checked on a regular schedule to determine mortalities. If bioaccumulation is being studied, several specimens are sacrificed at set time intervals over the study period. Specimens for analysis are handled and preserved as described in Subsection E3 of this appendix.

## C7. Miscellaneous Invertebrate Collection Techniques

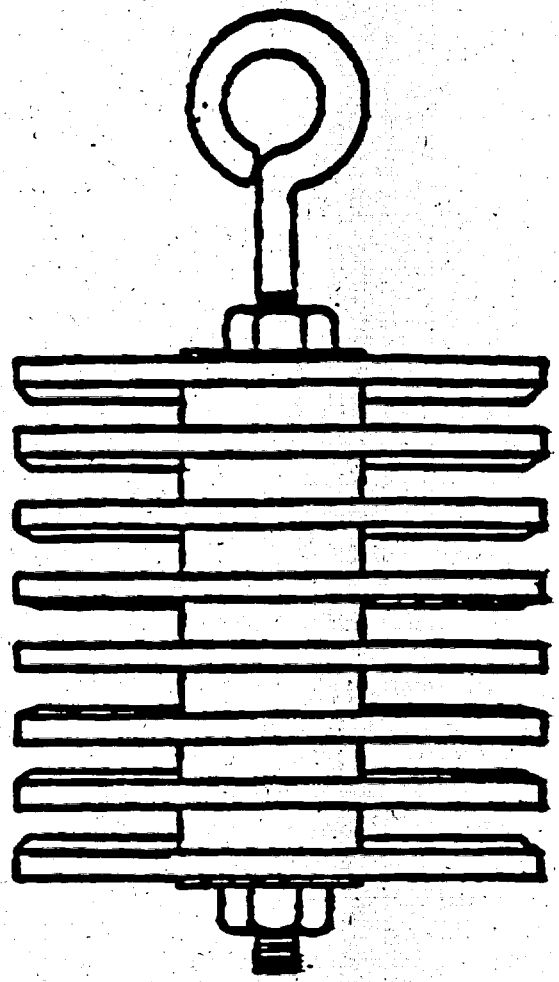
Aquatic invertebrates can be collected in a variety of other ways, depending on the species and habitats being sampled.

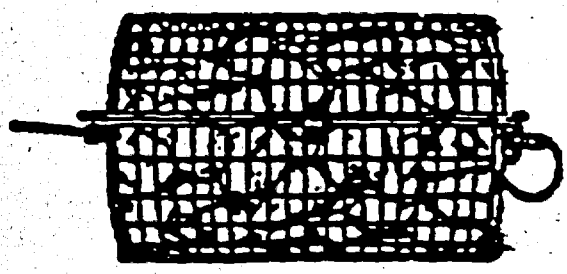
Other sampling devices that can be used include garden rakes, pocket knives, buckets, tongs, dip nets, and hands. Any of these methods would best be used for collection of organisms for tissue analysis and not for ecological surveys since they are difficult to quantify.

# D. FISH FIELD COLLECTION TECHNIQUES

Collection techniques for gathering biotic information on both freshwater and marine fish species include trawling, seining, hook and line, and in situ bioassays. Electroshocking is used in freshwater systems only.

Exhibit 12A-6
SUBSTRATE SAMPLERS





## D1. Trawls

The trawl method of sampling fish consists of dragging an open net through a body of water boat. The net is set at the appropriate operating depth to catch the species of interest. This sar method is used in large, open-water areas of reservoirs, lakes, rivers, estuaries, and oceans. Irregula toms or areas with snags or large debris items are difficult to sample by trawl. Otter trawls (Exhibit 1 are commonly used because they can be operated from a relatively small boat.

The otter trawl method is used to sample bottom species while midwater species are often sampl a modified otter trawl (beam trawl) system.

Because many pollutants concentrate in sediments, bottom trawling is useful in collecting orga that are associated with sediments. This sampling method can be used to collect specimens for analysis or for ecological surveys to describe comparative populations (i.e., potentially impacted are sus reference area). However, there are limitations to using trawls to describe the entire populatic cause some species are able to avoid being captured in the net.

Otter trawls are composed of two rectangular "otter boards" attached to the forward end of eact of the net. These boards are used to hold the mouth of the net open. The opening of the smaller tra about 16 to 20 feet. The length of line used to fish the trawl depends on the depth of the body of The preferred angle on the line is at least 5 feet of line per foot of depth. The net is a semi-balloon mo shrimp trawl with .75-inch mesh, and it often has an additional liner of .25-inch mesh in the end of th (cod end) to retain smaller fish. The bottom line of the net mouth is a lead line to keep the net fishir bottom, and the top line includes floats to keep the net open.

Small trawls can be operated by two people in a medium-sized power boat. While the trawl ca hauled in by hand, a winch is more useful, especially when the catch is expected to be large.

The length of time for fishing with the trawl depends on the expected abundance of organisms. time usually varies from 5 to 15 minutes and begins when the net starts fishing the bottom. After the hauled back on deck, specimens collected are handled as appropriate for the study program. A de discussion of fish handling and preservation techniques used in the field is included in Subsection D5 get Fish Species) of this appendix.

Other miscellaneous gear includes life jackets, wet-weather gear (even in dry weather), gloves, a containers to hold the catch into for sorting and examination. Before collecting fish with bottom traw some information is required to determine expected sediment contamination levels. Personal protect gear is used when necessary.

## D2. Electrofishing

Electrofishing is a freshwater fish sampling method that uses a pulsating direct current electroshocker, which stuns fish when the electric current travels through water with a resistance bet 300 ohms and 30,000 ohms. Alternating current (AC) or nonpulsing DC methods are available but are not as desirable because higher fish mortality occurs with AC. Pulsating DC often gives better results nonpulsing DC (Smith-Root, n.d.).

Electrofishers can readily be used to collect specimens for tissue analysis or to obtain populatic timates or other population factors for creeks or small rivers in ecological surveys. When using electr ing in ecological studies, several factors should be considered. These include size selectivity (large

Exhibit 12A-6
OTTER TRAWL

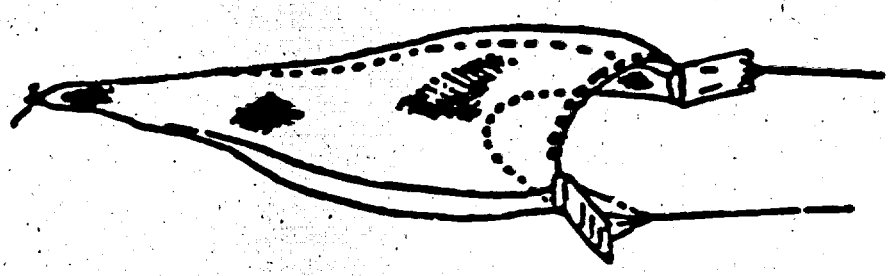
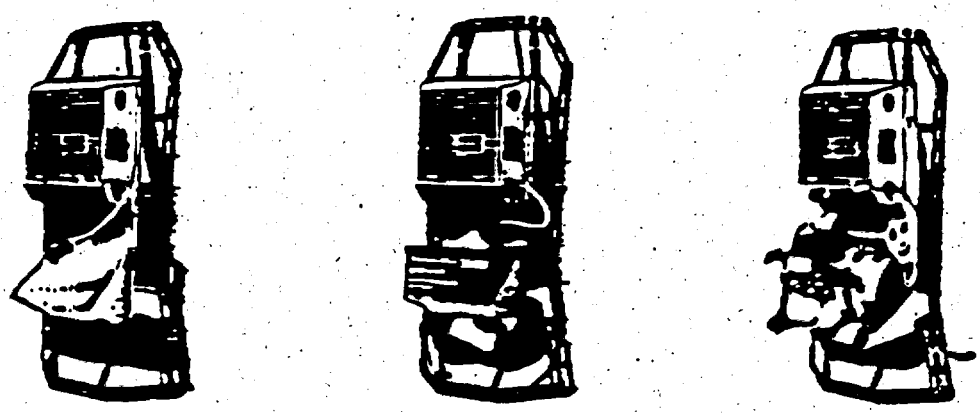
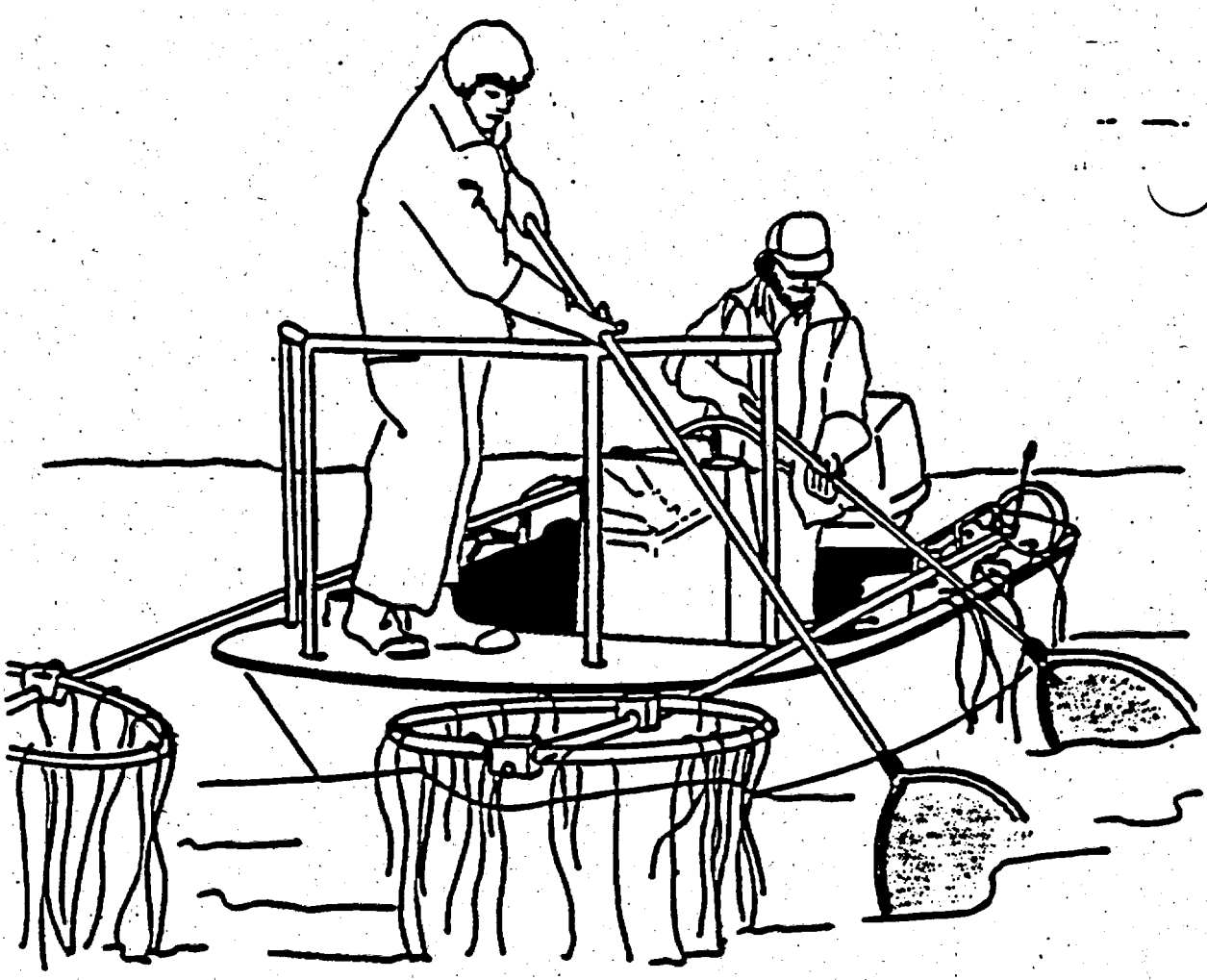


Exhibit 12A-7
BACKPACK AND BOAT ELECTROSHOCKERS





are more easily stunned than smaller fish); behavioral and habitat preference differences between species; and water conductivity, temperature, depth, and turbidity.

Electrofishing in creeks and small rivers can be done with a backpacking model pulsating DC electroshocker (top of Exhibit 12A-7). The backpacker system includes the electroshocker control unit and a 12-volt battery mounted in a specially designed backpack unit, the anode pole, and the cathode screens. The circular anode unit is mounted on a pole, which can be outfitted with a small mesh net to capture stunned fish. Other gear needed includes a long-handled, fine-mesh dip net to capture fish and a bucket to hold specimens. Because electroshockers have a high-voltage output, other important equipment includes nonleaking, chest-high wading boots and nonleaking rubber electrician's gloves with long cuffs. Life jackets are also worn.

Waters that cannot be waded in can be electrofished by boat (Exhibit 12A-7). The anode is clamped rigidly ahead of the boat and extends into the water. One person guides the boat with oars or a motor while one or two operators dip stunned fish. In waters too deep to wade, larger fish are more often taken by the boat method rather than by the backpack method. The same safety equipment is used in boat electro shocking as for the backpack method. The boat electroshocker is equipped with a "dead man" switch that allows for a quick disconnect of the electrical impulse if a person falls in the water.

Some knowledge of expected species and their suitable habitat is helpful in electrofishing. When stunned with a DC system, a fish will often be drawn toward the anode. However, in running waters, it can be swept downstream. Polarized dark glasses can aid in finding stunned fish. Collected organisms are placed in a water-filled bucket until processing can take place. Organisms to be used for tissue analysis are processed as described in Subsection D5 (Target Fish Species) of this appendix.

## D3. Seining

Seining involves the use of a long strip of netting hung between a float line and a lead-weighted line that is pulled through the water either by boat or, in shallow waters, by hand. This method is most often used in shallow sandy beach areas in either fresh or salt water. Beach seining is a simple sampling method that can collect fish samples for tissue analysis and can provide some information on species variability in ecological studies. Because certain sizes and types of fish can easily escape a beach seine, its use in ecological studies is limited.

A small beach seine consists of a nylon net equipped with cork or plastic floats on the top and a lead- or steel-weighted line on the bottom. The size of the net will depend on the area to be sampled, but a typical size is approximately 10 meters long and 3 meters deep. Mesh size can vary with the species of interest. Hauling lines are attached to the top and bottom lines by a short bridle. This type of small seine can be operated by two people. If the water is shallow, no boat is needed. One person anchors one side of the seine on the beach, while the other deploys the seine through the area to be fished. Both ends are then pulled on shore as quickly as possible, making sure that the bottom line remains on the bottom. Collected organisms are processed according to the study plan using techniques as described in Subsection D5—Target Fish Species of this appendix.

## D4. Hook and Line

Fishing with a hook and line involves the use of a hand-held rod or trolling baited hooks or other lures. While this method is not usually acceptable in ecological surveys, it is often the best way to obtain a few specimens for chemical analysis when other methods are not possible. Occasionally, fish freshly caught by

nonstudy-team personnel are used in tissue analysis studies if enough information is known regarding the location of catch. This study can also provide information regarding human consumption of local species.

## D5. In Situ Bioassay

Local fish species can be used in field bioassays in the same manner as was described for macroinvertebrates in Subsection C3 of this appendix.

### Target Fish Species

Before sampling fish for tissue analysis, the study team identifies possible target species based on the following:

- Geographic location
- Available habitat
- Ease of capture and identification
- Pollution tolerance
- Use as a sport fish
- Nonmigratory habits

Exhibit 12A-8 lists possible target species by geographic location. While trout are identified as one of the preferred target fish species, caution is exercised in using these fish because in many areas, especially in the east, trout are stocked on a "put and take" basis. The local agency responsible for stocking can be contacted to determine when fish were stocked in a particular area. A period of 3 months is considered to be the minimum time span for trout to acquire a reasonable concentration of ambient contaminants (Freed et al., 1980).

The season during which fish are collected for tissue analysis is also an important consideration. The spawning season should be avoided whenever possible because fish are often stressed during this time; they also have different feeding habits, fat content, and respiration rates, which can influence pollutant uptake and clearing.

Exhibit 12A-8
TARGET FISH SPECIES FOR USE IN TISSUE ANALYSIS

I. Target Species (East of Appalachian Mountains)

***Brook Trout (*Salvelinus fontinalis*)
***Small Mouth Bass (*Micropterus dolomieui*)
***Large Mouth Bass (*Micropterus salmoides*)
***Channel Catfish (*Ictalurus punctatus*)
**Brown Trout (*Salmo trutta*)
**Rainbow Trout (*Salmo gairdneri*)
**Bluegill (*Lepomis macrochirus*)
**Pumpkinseed (*Lepomis gibbosus*)
**Black Crappie (*Pomoxis nigromaculatus*)
**Striped Bass (*Morone saxatilis*)
*Carp (*Cyprinus carpio*)

II. Target Species (West of Appalachian Mountains and East of Rocky Mountains)

***Rainbow Trout (*Salmo gairdneri*)
***Brook Trout (*Salvelinus fontinalis*)
***Small Mouth Bass (*Micropterus dolomieui*)
***Large Mouth Bass (*Micropterus salmoides*)
***Channel Catfish (*Ictalurus punctatus*)
**Striped Bass (*Morone saxatilis*)
**Yellow Perch (*Perca flavescens*)
**Walleye (*Stizostedion vitreum*)
**Bluegill (*Lepomis macrochirus*)
*Brown Trout (*Salmo trutta*)
*Carp (*Cyprinus carpio*)

III. Target Species (West of and Including Rocky Mountains)

***Rainbow Trout (*Salmo gairdneri*)
***Brook Trout (*Salvelinus fontinalis*)
***Small Mouth Bass (*Micropterus dolomieui*)
***Large Mouth Bass (*Micropterus salmoides*)
***Channel Catfish (*Ictalurus punctatus*)
**Bluegill (*Lepomis macrochirus*)
**Striped Bass (*Morone saxatilis*)
*Cut-throat Trout (*Salmo clarki*)
*Brown Trout (*Salmo trutta*)
*Carp (*Cyprinus carpio*)

*** Preferred target species
** Good target species
* Acceptable target species

Source: Freed et al., 1980.

## E. BIOLOGICAL FIELD SAMPLE PROCESSING AND PRESERVATION TECHNIQUES

### E1. Vegetation

Samples of vegetation collected from the site and intended for classification are initially placed in a ridged collector's box. Samples should be kept moist and may be refrigerated when the collector returns to the laboratory. After identification, samples may be pressed and mounted for permanent records.

Vegetation samples collected for tissue analysis are placed in 1-gallon paper bags and labeled. Information on the label includes the date, time, weather, collector, plant type, site, identification number, and proposed analysis.

The bag is stapled or clipped shut, labeled with the identification numbers, and placed in a larger plastic bag. Several paper bags may be necessary to collect 30 grams of material; 1 gram of plant tissue can suffice for most analyses that require the same analytical processing. However, more than 30 grams should be taken if multiple testing or other special processing are required. The plastic bag is then placed in a cooler with ice, ice packs, or dry ice. Care is taken to keep cooler water from contacting collected plant material. Samples remain in coolers for shipment.

### E2. Terrestrial Vertebrates

Once the specimens are collected, organisms to be used for tissue analysis are killed. Each animal described by weight, measurement, sex, and other general items. All specimens are photographed. The following tissues can be removed using stainless steel scalpels: muscle and associated fatty deposits (lipids), liver, kidneys, and possibly hair and claw samples for metal analysis. Stomach or crop contents can be removed and preserved for identification. Any anomalies are noted and photographed. Sections of the anomalous tissues may be taken for analysis. Tissues are immediately wrapped in cleaned aluminum foil (dull side in), labeled, and frozen in the field using dry ice. Hair and claw samples are placed in plastic bags and labeled. Tissue samples are kept frozen until they are delivered to the laboratory. Surgical gloves are used during the dissecting process.

### E3. Aquatic Macroinvertebrates

Invertebrates collected for tissue analysis are sorted by species, counted, measured (when appropriate), and weighed to assure that each single sample consists of at least 100 grams. Crustaceans are washed using distilled water to remove particulate matter, either wrapped in cleaned aluminum foil (dull side in) or placed in Contract Laboratory Program cleaned glass vials, labeled, and frozen using dry ice. Samples are packed in ice chests and kept frozen until they are delivered to the laboratory. Bivalve mollusks are removed from their shells with a stainless steel knife for the examination of organochlorine compounds or with a plastic knife for the examination of metals. Tissues are purged using distilled water, wrapped in aluminum foil (dull side in), labeled, and frozen as described above. For organic analysis, organic-free water and blanks should be employed to document contamination control. Surgical gloves are worn while handling invertebrate samples. Glove manufacturers should be contacted to determine if gloves are a source of contamination and, if so, what compounds are typical.

## Exhibit 12A-9
## FISH PROCESSING PROCEDURES

1. Wash fillet board and table with local water supply (river, lake, etc.). Distilled water may be used.

2.** Clean knives with acetone and wipe board and table with acetone rinse. Rinse all with local water supply or distilled water.

3. Rinse table and knives between specimens with distilled water; alternately, a previously cleaned knife (#2 above) can be used for each specimen.

4. Take scale sample just posterior of gill and place in scale envelope.

5. Take weight (kg) and length (mm), and record on data sheets.

6. Fillet according to Exhibit 12A-11.

7. Wrap fillets in aluminum foil, and secure with 2-inch masking tape.

   a. If large, individually
   b. If small, combine

8. Label package with

   a. Date and time of collection and preparation
   b. Location (river, lake, etc.)
   c. Species
   d. Sample number
   e. Project number
   f. Sampler's/preparer's name

9. Place in bag and store in ice chest with dry ice.

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**Note: If volatile analyses are required, acetone use is discouraged and methanol can be used as a substitute.

Source: Michigan Department of Natural Resources.

## Exhibit 12A-10
## FISH COLLECTION EQUIPMENT CHECKLIST

Fillet table (formica top with long legs)
Wrapping table (aluminum, folding)
Fillet knives (1 large and 1 small)
Steel
Clear plastic packaging bags
Garbage bags
Fillet boards--polycarbonate
Water bucket
Wash brush
Garbage pail--6-gallon plastic or wastebasket
Data sheets
Procedure forms--fillet technique, skin-on or skin-off
Plastic bag ties
Scissors
2-inch masking tape
Marking pens and pencil
24-inch-wide roll of heavy-duty aluminum foil
Fish-scale envelope
Tripod
Fish-spring scale
Fish-measuring board
Ice chests
Paper towels
Acetone (wash bottle)**
Dry ice

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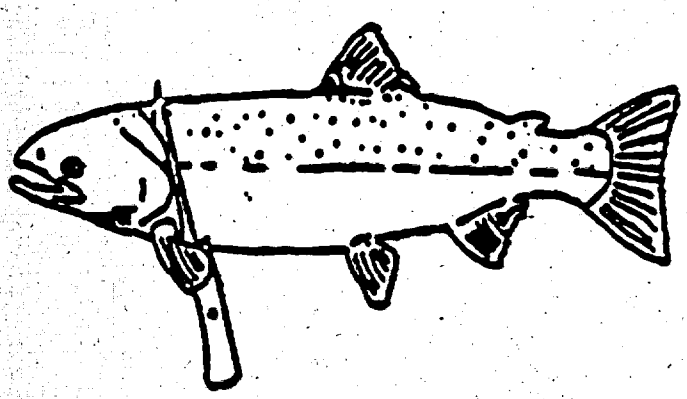
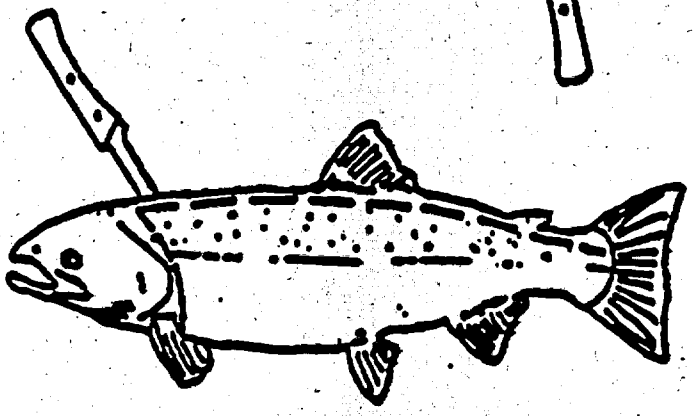
**May need to substitute methanol (see Exhibit 12-9).

Source: Michigan Department of Natural Resources.

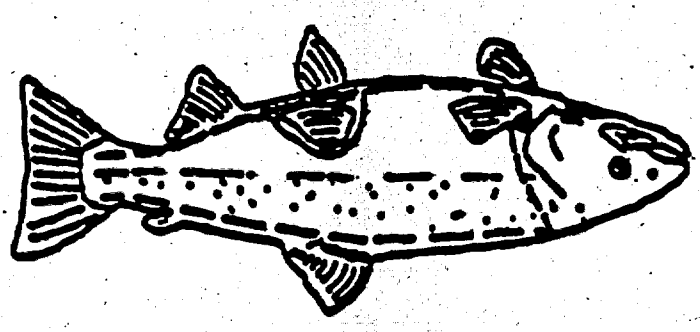
## Exhibit 12A-11
## PREPARATION OF "STANDARD FILLETS"

1. Make a cut behind the entire length of the operculum (gill cover) cutting through the skin and flesh to the spinal column.

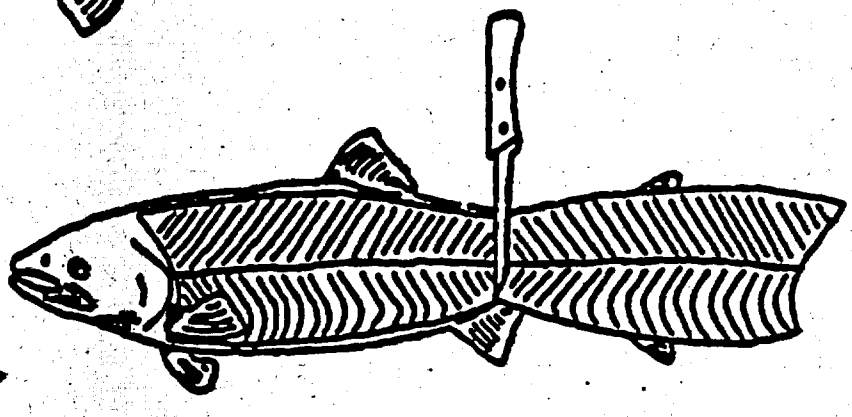




2. Make a shallow cut through the skin (on either side of the dorsal fin) from the base of the caudal peduncle.



3. Make a cut along the belly from the base of the pectoral fin to the posterior end of the caudal peduncle. This cut is made on both sides of the anus and the anual fin.



4. Remove the fillet and then remove any major bones.

Exhibit 12A-12
STANDARD EDIBLE PORTIONS OF SELECTED
SPORT AND COMMERCIAL FISH

| Standard Edible Portion | Common Names | Scientific Name |
|---|---|---|
| Skin-on Fillet (all below to next heading) | Yellow perch | *Perca flavescens* |
| | Walleye | *Stizostedion vitreum* |
| | Sauger | *Stizostedion canadense* |
| | Largemouth bass | *Micropterus salmoides* |
| | Smallmouth bass | *Micropterus dolomieui* |
| | Bluegill | *Lepomis macrochirus* |
| Skin-on | Pumpkinseed | *Lepomis gibbosus* |
| | Rock bass | *Ambloplites rupestris* |
| Fillet | White perch | *Morone americana* |
| | Black crappie | *Pomoxis nigromaculatus* |
| | White crappie | *Pomoxis annularis* |
| | Green sunfish | *Lepomis cyanellus* |
| | Longear sunfish | *Lepomis megalotis* |
| | Warmouth | *Lepomis gulosus* |
| | Sucker family | *Catostomidae* |
| | Lake whitefish | *Coregonus clupeaformis* |
| | Lake trout | *Salvelinus namaycush* |
| | Rainbow trout | *Salmo gairdneri* |
| | Brown trout | *Salmo trutta* |
| | Brook trout | *Salvelines fontinalis* |
| | Splake | *Salvelinus poticalis** |
| | Lake trout | *Salvelinus namaycush* |
| | Atlantic salmon | *Salmo salar* |
| | Coho salmon | *Oncorhynchus kisutch* |
| | Chinook salmon | *Oncorhynchus tshawytscha* |
| | Pink salmon | *Oncorhynchus gorbuscha* |
| | Black bullhead** | *Ictalurus melas* |
| | Brown bullhead** | *Ictalurus nebulosus* |
| | Yellow bullhead** | *Ictalurus natalis* |
| | Channel catfish | *Ictalurus punctatus* |
| | Muskellunge | *Esox masquinongy* |
| | Northern pike | *Esox lucius* |
| | Round whitefish | *Prosopium cylindraceum* |

Exhibit 12A-12
(continued)

| Standard Edible Portion | Common Names | Scientific Name |
|---|---|---|
| Skin-off Fillet | Lake herring (cisco) | *Coregonus artedii* |
| (all below to next heading) | Chubs (bloater) | *Coregonus hoyi* |
| | Carp | *Cyprinus carpio* |
| | Freshwater drum | *Aplodinotus grunniens* |
| | Bigmouth buffalo | *Ictiobus cyprinellus* |
| | Burbot | *Lota lota* |
| | Quillback | *Carpiodes cyprinus* |
| | Lake sturgeon | *Acipenser fulvescens* |
| Headless, gutted, whole fish | Rainbow smelt | *Osmerus mordax* |

* Hybrids between brook trout (*S. fontinalis*) and lake trout (*S. namaycush*) are known as splake.

**Depending on local consumptive practice, bullheads may be considered "skin-off" species since they are skinned before consumption.

Source: Modified from Michigan Department of Natural Resources.

Invertebrates collected for ecological assessment are preserved in the field with either a 4- to 7-percent formalin solution (dependent on sample use and fragile nature of animals) or with 70-percent buffered ethanol. Each sample is labeled with sampling location, depth, sample number, species (or lowest taxonomic level practicable), number of individual organisms collected, sampling method, date, project number, sampler, and team leader.

## E4. Fish

Fish collected for tissue analysis are handled according to procedures outlined in the Michigan Department of Natural Resources (MDNR) Fish Processing Procedures (Exhibit 12A-9) and in the Field Collection Equipment Checklist (Exhibit 12A-10). Exhibit 12A-11 shows the procedure for preparation of MDNR's "standard fillets," and Exhibit 12A-12 lists the standard edible portions of selected sport and commercial fish. Any marine fish that may be associated with a hazardous waste site and is not on this list will require input from local individuals as to which tissues are consumed. Other fish tissues (i.e., liver, bone, etc.) may need to be analyzed depending on contaminants involved and where they may accumulate.

Attachment 2

# Fish Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters

EPA/600/R-92/111
March 1993

# FISH FIELD AND LABORATORY METHODS FOR EVALUATING THE BIOLOGICAL INTEGRITY OF SURFACE WATERS

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## SECTION 11

## GUIDELINES FOR FISH SAMPLING AND TISSUE PREPARATION FOR BIOACCUMULATIVE CONTAMINANTS

### 11.1 Introduction

11.1.1 Sampling of fish and shellfish for bioaccumulative contaminants has been conducted for over 35 years. Most fish sampling for contaminants has focused on contaminants of local concern, so data results and program conclusions have not always been comparable. The issues surrounding management of chemical contaminants in fish are of increasing concern for fishery management, environmental and public health agencies. The interdisciplinary multiagency problems caused by chemical contaminants suggests the need for standard sampling protocols. There have been inconsistent warnings given to the public by local, state, and federal regulatory agencies regarding the consumption of sport fish. This has been particularly evident on bodies of water shared by two or more states and on international waters. The Great Lakes States (Great Lakes Fish Consumption Advisory Task Force) and those States and EPA Regions bordering the Mississippi (Mid-America Fish Contaminants Group) and Ohio Rivers (Ohio River Valley Water Sanitation Commission) have endeavored to provide consistent sampling and advisory information but a standard protocol has yet to be agreed upon.

11.1.2 The application of quantitative risk assessment including hazard assessment, dose response assessment, exposure assessment and risk characterization functions best with a standardized protocol. The development of human health fish consumption advisories, whether based on quantitative risk assessment or some other methodology, is fundamentally affected by the procedures used in sampling. This section presents guidance for the sampling and preparation of fish for contaminant analysis, which is a key component of exposure assessment in quantitative risk assessment.

11.1.3 The purpose and goals of each study should be clearly stated prior to the initiation of fish collection for contaminant analysis. One should consider the overall long-term development of a fish contaminant database in each jurisdiction. Frequently short term goals have been the only consideration, where as long term trend assessments may provide a better understanding of the problem because the long view is the only way of gauging important changes occurring in water quality.

11.1.4 Various federal, state, and local agencies have responsibilities for the collection and preparation of fish samples. Thus, numerous collection protocols are available. Fish sampling for contaminant analysis will often be included in other biological surveys to maximize use of the resource and to minimize costs. It must be recognized that any sample collected represents the future expenditure of significant dollar amounts by the time a decision is reached, and can have significant effects on major sectors of our society.

11.1.5 These guidelines present a basic fish sampling protocol designed to give comparable results between studies. Some additional requirements are pointed out which may be needed in special studies where different sizes or species of fish might be targeted or where special collections for spike samples might be needed. A partial discussion of sampling strategy including statistical concerns can be found in USEPA (1989), which should be reviewed during any planning effort.

11.2 Site Selection

11.2.1 Collecting sites should be established according to the specific requirements of each study. Sites may be designed as short- or long-term depending on the frequency with which they are sampled. Most sampling designs for short-term (synoptic) studies will be structured to determine the extent of contamination in a water body or a section of a water body. The determination of contamination gradients extending away from point sources or industrial/urban areas with point and non-point sources provides important information needed to manage contaminant burdens in fish. Some sites will be selected by individual states to address intrastate needs while other sites will be selected to address interstate needs through cooperative programs. Regardless of the various reasons for site selection, long-term comparability is of utmost importance to provide trend information needed to place bioaccumualtive contaminants in perspective.

11.2.2 Sites should be described as sport, commercial, or having both types of fisheries, and additional sites may be identified for ecological risk assessment. Special watershed information should be indicated, including urban areas, mining, manufacturing, agriculture, etc., and any known point or non-point sources of pollution at or near the site in the watershed. Additional information should include average width, depth, and velocity at the sampling station, description of the substrate, duration of the sampling effort, and habitat area sampled (e.g., length of stream or area of lake). Selected water quality measurements (e.g., conductivity, pH, dissolved oxygen, temperature, etc.) may also be useful. It is becoming routine to collect and analyze water, sediment and fish at common stations to gain a more complete understanding of contaminants in aquatic environments.

11.3 Sample Collection

11.3.1 The following three objectives should guide sample collection:

1. Provide comparable data

2. Utilize sizes and ages of species generally available to the fishery and,

3. Yield data which will screen for problems that might indicate that more intensive studies are needed.

11.3.2 Samples should be obtained at each station from the principal fish categories. Fish species are grouped by feeding strategy into predators, omnivores and bottom feeders. To reduce the number of categories, the

omnivores may be placed with the bottom feeders. USEPA (1990a) sampled 388 sites nationwide at which 119 different species of fish representing 33 taxonomic families of fish were collected. The most frequently sampled freshwater and marine species in that study are listed in Table 1.

11.3.3 This national study indicates that of the freshwater species, carp and largemouth bass were the most frequently sampled and are the most likely to provide interstate comparability. The other freshwater species listed may be selected in a declining order of priority; however, additional less common species may not be added except in special situations. The diversity of marine species is much greater resulting in a lack of focus on a limited number. Additional effort will be needed to determine which marine species should receive priority on the Atlantic, Pacific and Gulf Coasts in order to provide long term comparative data.

11.3.4 Cunningham et al. (1990) in a census of state fish/shellfish consumption advisory programs found that approximately 60 species of fish and shellfish are used as the basis for consumption advisories nationwide. The leading fish families are the Ictaluridae (catfish), Centrarchidae (sunfish, largemouth and smallmouth bass), Cyprinidae (carp), and Salmonidae (salmon and trout). Among shellfish, crustaceans (e.g., blue crab) and molluscs (e.g., American oyster, soft-shelled clam, and blue mussel) are the most widely used. The criteria most frequently used for collecting fish/shellfish species were: 1) the dominant species harvested for consumption, 2) the most abundant species and 3) the species representing a specific trophic order.

11.3.5 Consistent sampling of common species over long time periods (several years) and large geographic areas will greatly facilitate future trend analyses. Many species are similar in appearance, and taxonomic identification must be reliable to prevent mixing species. Under no circumstance should two or more species be mixed to create a composite sample. Fish for contaminant analyses may be obtained during studies to determine fish community structure. The measurement of multiple parameters (e.g., fish health condition assessment, histopathological examination, bioindicators of stress, etc.) are encouraged on common samples to provide the information needed in ecological risk assessment.

11.3.6 Screening studies should endeavor to collect the largest individuals available. However, more detailed studies should sample the predominant two or three age classes of the same species in a water body to determine the relationship between contaminant burden and fish size (age) to provide information needed for greater risk management flexibility. This information could allow the lifting of an advisory on smaller, more abundant sizes of a contaminated species with lower body burdens if these were important to a sport fishery.

11.3.7 The frequency of sampling should be considered in each study design. Most long-term monitoring programs will be based on an annual frequency due to the costs of analysis. However, special studies may require seasonal sampling. Fish sampled in the fall may tend to have a higher lipid content than those sampled during the spring. Sampling freshwater in the spring may

TABLE 1. FREQUENCY OF OCCURRENCE FOR FRESHWATER AND MARINE SPECIES IN THE NATIONAL FISH BIOACCUMULATION STUDY (USEPA, 1990a)

FRESHWATER

| Bottom Feeder Species | Site Occurrence |
|---|---|
| Carp | 135 |
| White sucker | 32 |
| Channel Catfish | 30 |
| Redhorse sucker | 16 |
| Spotted sucker | 10 |

| Game (Predator) Species | Site Occurrence |
|---|---|
| Largemouth Bass | 83 |
| Smallmouth Bass | 26 |
| Walleye | 22 |
| Brown trout | 10 |
| White Bass | 10 |
| Northern Pike | 8 |
| Flathead Catfish | 8 |
| White Crappie | 7 |
| Rainbow trout | 7 |

MARINE

| Species | Site Occurance |
|---|---|
| Hardhead catfish | 7 |
| Starry flounder | 5 |
| Blue fish | 5 |
| White perch | 4 |
| Winter flounder | 4 |
| White sturgeon | 4 |
| Red drum | 3 |
| Black drum | 3 |
| Striped mullet | 3 |
| Atlantic croaker | 3 |
| Spot | 3 |
| Spotted seatrout | 3 |
| Weakfish | 3 |
| Sheepshead | 2 |
| Southern flounder | 2 |
| Flathead sole | 2 |
| Atlantic salmon | 2 |
| Red snapper | 2 |
| Gizzard shad | 1 |
| Atlantic cod | 1 |
| Yellow jack | 1 |
| Striped bass | 1 |
| American shad | 1 |
| Surf smelt | 1 |
| Spotted drum | 1 |
| Crevalle jack | 1 |
| Redstripe rockfish | 1 |
| Summer flounder | 1 |
| Diamond turbot | 1 |
| Hornyhead turbot | 1 |
| Bocaccio | 1 |
| White surfperch | 1 |
| Quillback rockfish | 1 |
| Brown rockfish | 1 |
| Copper rockfish | 1 |
| American eel | 1 |

find fish more available due to spawning movements exhibited by spring spawning species; however, extensive movement may temporarily dislocate fish from the usual area where they have been exposed to contaminants. The various methods of collecting fillets (skin-on versus skin-off, belly flap included or excluded) must be standardized. A skin-on fillet with belly flap included is recommended. A lipid analysis of each sample is required for trend analysis and model validation, however, lipid content is not recommended for use in normalizing the differences among fillet types because it frequently increases the variance in the data (NOAA, 1989). Even when considering the bioaccumulation of lipophilic compounds all of the compound is typically not stored in the lipid. At any given time additional amounts of the compound will be found in the cell moisture and the non-lipid tissue. Lipid content may also provide insight into seasonal changes within species, as well as identify differences between species used in contaminants monitoring.

11.3.8 Active sampling techniques (electrofishing, trawling, seining, etc.) are preferred over passive capture techniques (gill nets, trammel nets, etc.) however, the latter can be used as long as the gear is checked on a frequent basis to avoid sample deterioration. Species that are difficult to collect may be obtained from a commercial fisherman, but only when the collector accompanies the fisherman to verify the time and place of capture. Following collection, fish should be placed on wet ice in clean coolers prior to processing. Fish should be either processed within 24 hours or frozen within 24 hours for later processing if immediate processing is not possible. If analyses of fish eggs or internal organs are required, a sample size of at least 20 grams is required.

11.3.9 Composite samples of three to ten fish (same species) are recommended for each of the predator and bottom feeder categories based on the variability of contaminant concentration in fish at the site. The number of fish/composite selected should remain constant over time and space for each species monitored. Composites are used to reduce the cost of analysis per fish; however, it must be recognized that statistical manipulation of the data is compromised when individual values are not determined. The smallest size fish in a composite should equal 75% of the total length of the largest fish in a composite, e.g., if the largest is 400 mm, the smallest should not be less than 300 mm. Replicate composite samples may be added as needed to meet statistical requirements; (USEPA, 1989) however, the cost of additional samples will quickly become a factor. The most important sport and/or commercial species in each feeding strategy group should be used for analysis. Composite samples can be collected for either fillet analysis (human health risk assessments) or for whole body analysis (ecological risk assessments and worst case monitoring). Each composite should contain 200 g of tissue so sufficient material is available for analysis of recommended target analytes.

11.3.10 When a study is planned, it is not certain that the quantity of each species indicated for analysis can be obtained especially if the water body has had little or no prior sampling activity. In order to meet both the human health and ecological requirements a sample of a sport fish species and a bottom feeder species is needed. The sport fish species is usually filleted and the data used for human health risk assessment. The whole body analysis of bottom feeder species is used both for initial "worst case" monitoring and for ecological risk assessment.

11.3.11 If fish are not abundant or detailed comparisons with other parameters are desired, it may be possible to do a reconstructed analysis (Figure 1) on a single species either sport fish or bottom feeder. To do a reconstructed analysis, the fish are filleted and the remainder of the carcass is saved for analysis. The contaminant concentrations in both the fillet and remaining carcass portions can then be added together to estimate the whole body concentration. A lipid analysis must be performed on both the fillet and remaining carcass to allow normalization of the contaminant concentrations in both samples. A reconstructed analysis may be performed on either single fish or composite fish samples, however, the data may be more reliable if single fish are analyzed.

11.3.12 Sediment samples can sometimes indicate a "hot spot" and can be helpful in determining the source(s) of contamination or the zones of deposition. However, sediment samples cannot be used as a substitute for fish collections, but both can provide complimentary data.

11.4. Sample Preparation For Organic Contaminants in Tissue

11.4.1 Collection Precautions

11.4.1.1 In the field, sources of tissue contamination include sampling gear, boats and motors, grease from ship winches or cables, engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. For example, to avoid contamination from ice, the whole samples (e.g., molluscs in shell, whole fish) should be wrapped in aluminum foil, placed in watertight plastic bags, and immediately cooled in a covered ice chest. Many sources of contamination can be avoided by resecting (i.e., surgically removing) tissue in a controlled environment (e.g., a laboratory). Organisms should not be frozen prior to resection if analyses will be conducted on only selected tissues (e.g., internal organs) because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated in the field, the remaining tissue may be wrapped as described above and frozen. Tissue sample collection and preparation requirements are summarized in Table 2 (Puget Sound Estuary Program, 1989).

11.4.2 Processing

11.4.2.1 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, rinsed in isopropanol, and finally rinsed with organic free distilled water. Work surfaces should be cleaned with isopropanol, washed with distilled water and allowed to dry completely.

11.4.2.2 The removal of biological tissues should be carried out by or under the supervision of an experienced biologist. Tissue should be removed with clean stainless steel or quartz instruments (except for external surfaces). The specimens should come into contact with precleaned glass surfaces only. Polypropylene and polyethylene (plastic) surfaces and implements are a

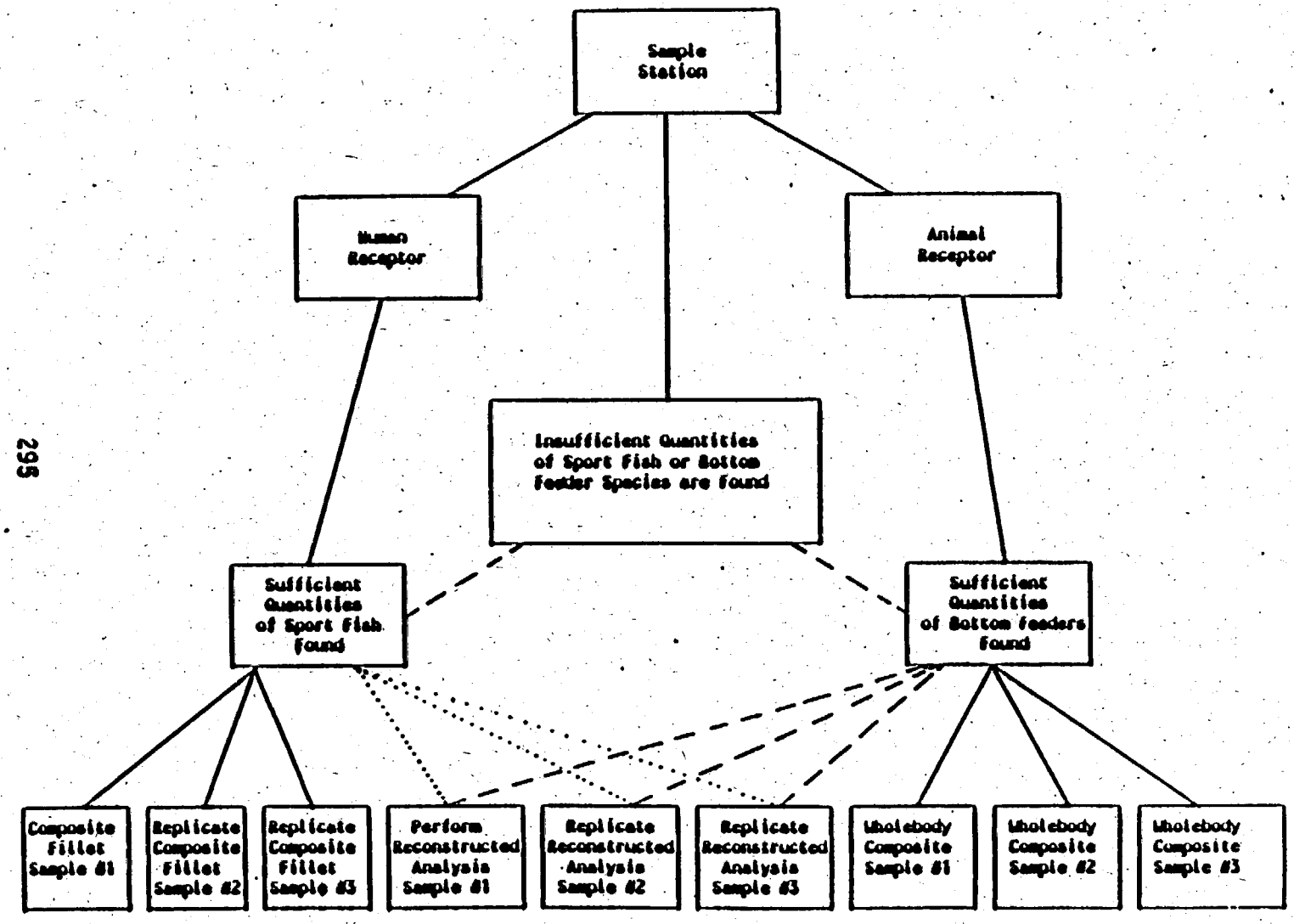


Figure 1. General sampling scheme for bioaccumulative contaminants in fish, multiple age groups will require additional samples.

TABLE 2. SUMMARY OF SAMPLE COLLECTION AND PREPARATION QA/QC REQUIREMENTS FOR FISH TISSUE (MODIFIED FROM PUGET SOUND ESTUARY PROGRAM, 1986, 1989)

| Variable | Sample Size (a) | Container (b) | Preservation | Maximum Holding Time (c) | Maximum Extract Holding Time |
|---|---|---|---|---|---|
| **Organic Compounds** | | | | | |
| Wholebody Tissues (after resection) | -- | A | Freeze (-18°C) | 1 yr | 40 days |
| Semivolatiles | 25 g | G,T,A | Freeze (d) (-18°C) | 1 yr | 40 days |
| Volatiles | 5 g | G,T | Freeze (d) (-18°C) | 14 days | -- |
| **Trace Metals** | | | | | |
| Wholebody Tissues (after resection) | -- | W,P,B | Freeze | 6 mo | |
| All Metals | 5 g | P,B | Freeze (d) | 6 mo | |
| (except Hg) | 0.2 g | P,B | Freeze (d) | 28 days | |

a. Recommended wet weight sample sizes for one laboratory analysis. If additional laboratory analyses are required (i.e., replicates) the field sample size should be adjusted accordingly. If specific organs are to be analyzed, more tissue may be required.

b. G = glass, A = wrapped in aluminum foil, placed in watertight plastic bags, T = PTFE (Teflon), P = linear polyethylene, B = borosilicate glass, W = watertight plastic bags.

c. This is a suggested holding time. No USEPA criteria exist for the preservation of this variable.

d. Post-dissection

potential source of contamination and should not be used. To control contamination when resecting tissue, technicians should use separate sets of utensils for removing outer tissue and for resecting tissue for analysis.

11.4.3 Preparation of Composite Fillet Samples

11.4.3.1 For fish samples, special care must be taken to avoid contaminating targeted tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The proper handling in the preparation of fish tissue samples to decrease the likelihood of contamination cannot be over emphasized. To reduce variation in sample preparation and handling, samples should be prepared in the laboratory rather than in the field. However, if no laboratory is available, field preparation is acceptable if portable tables are used, dust and exhausts are avoided and proper decontamination procedures are followed. Regardless of where preparation occurs, the following subsections should be followed to insure quality fillet samples:

11.4.3.2 To initiate processing, each fish is measured (total or fork length) to the nearest tenth of a centimeter, weighed (nearest gram) and external condition noted. A few scales should be removed from each fish for age and growth analysis. This presents an excellent opportunity to systematically evaluate each fish using the Fish Health and Condition Assessment Methods (Section 10). Fish are scaled (or skinned: catfish) and filleted carefully, removing bones, to get all of the edible portion flesh.

11.4.3.3 A fillet includes the flesh tissue and skin from head to tail beginning at the mid-dorsal line from the left side of each fish and including the belly flap. The fillet should not be trimmed to remove fatty tissue along the lateral line or belly flap. A comparable fillet can be obtained from the right side of the fish and can be composited with the left fillet, kept separate for duplicate quality assurance analysis, analyzed for different compounds or archived. Each right and left fillet should be weighed individually, recorded and individually wrapped in clean aluminum foil.

11.4.3.4 Care must be exercised not to puncture any of the internal organs. If the body cavity is entered, rinse the fillet with distilled water. Fish sex and condition of internal organs are determined during or after filleting. This skin-on fillet deviates from the skin-off fillets analyzed in the National Fish Bioaccumulation Study (USEPA 1990a), however, skin-on is recommended because it is believed that this is the way most sport anglers prepare their fillets. The issue of skin-on versus skin-off fillets differs greatly among jurisdictions (Hesse, 1990) and is far from settled, however, the above recommendations appear to be the preferred method unless the species specificity is increased in future guidelines.

11.4.3.5 Filleting should be conducted on cutting boards covered with heavy duty aluminum foil, which is changed between composite samples. Knives, fish scalers, measurement boards, scales, etc. should be cleaned with reagent grade isopropanol, followed by a rinse with distilled water between each composite sample.

11.4.3.6 Because of the low limits of detection for many environmental analyses, clean field and laboratory procedures are especially important. Sample contamination can occur during any stage of collection, handling, storage or analyses. Potential contaminant sources must be known and steps taken to minimize or eliminate them.

11.4.3.7 Large sheets of heavy duty aluminum foil should be used to carefully fold and completely wrap the fillet samples. When filling out I.D. labels use pencil or waterproof marker and place the foil wrapped sample in a secured plastic bag.

11.4.4 Storage

11.4.4.1 Recommended holding times for frozen tissue samples have not been established by USEPA, but a maximum 1 year holding time is suggested. For extended sample storage, precautions should be taken to prevent desiccation. National Institute For Standards and Technology is testing the effects of long-term storage of tissues at temperatures of liquid nitrogen(-120° to -190°C). At a minimum, the samples should be kept frozen at -20°C until extraction. This will slow biological decomposition of the sample and decrease loss of moisture. Liquid associated with the sample when thawed must be maintained as part of the sample because the lipid tends to separate from the tissue. Storage of samples should remain under the control of the sample collector until relinquished to the analytical laboratory.

11.4.4.2 Whole fish may be frozen and stored if no resection of internal organs or fillets will be conducted and the ultimate analysis is whole body. However, if resection of fillets or organs is required, these tissues should be removed prior to freezing and can be stored frozen in appropriate individual containers. The tissues may then be ground and homogenized at a later date and refrozen in sample packets for shipment on dry ice to the analytical laboratory(s).

11.4.4.3 It is frequently necessary to ship whole fish, fillets or homogenized tissue samples over long distances to an analytical laboratory. To avoid sample deterioration, it is recommended that all samples be frozen solid prior to shipment. The frozen and logged samples should be wrapped in newspaper to provide additional insulation for the samples which are shipped in well sealed insulated containers with an appropriate quantity of dry ice. The quantity of dry ice should be sufficient to eliminate any defrosting of the samples during the time of priority transport. However, in the event that a delay occurs in transit, these recommendations will provide some assurance that the samples will arrive in usable condition. Under no circumstances should unfrozen tissue be shipped either with or without dry ice because the quality of the sample cannot be assured.

11.4.5 Tissue Preparation

11.4.5.1 Organic contaminants are not evenly distributed throughout biological tissue, especially in fish. This is also true for fish fillets. Therefore, to obtain a homogenous sample, the whole fish or the whole fillet

weigh duplicate-1 gm portions into culture tubes with screw caps. Analyze immediately or store in a freezer.

## 11.5 Sample Preparation For Metal Contaminants In Tissue

### 11.5.1 Collection Precautions

11.5.1.1 The major difficulty in trace metal analyses of tissue samples is controlling contamination of the sample after collection. In the field, sources of contamination include sampling gear, grease from winches or cables, engine exhaust, dust, or ice used for cooling. Care must be taken during handling to avoid these and any other possible sources of contamination. For example, during sampling the ship should be positioned such that the engine exhausts do not fall on deck. To avoid contamination from melting ice, the samples should be placed in watertight plastic bags.

11.5.1.2 Sample resection and any subsampling of the organisms should be carried out in a controlled environment (e.g., dust-free room). In most cases, this requires that the organisms be transported on ice to a laboratory rather than being resected in the field. It is recommended that whole organisms not be frozen prior to resection if analyses will be conducted only on selected tissues, because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated in the field, the remaining tissue (e.g., muscle) may be wrapped as described above and frozen (Puget Sound Estuary Program, 1986).

11.5.1.3 Resection is best performed under "clean room" conditions. The "clean room" should have positive pressure and filtered air and also be entirely metal-free and isolated from all samples high in contaminants (e.g., hazardous waste). At a minimum, care should be taken to avoid contamination from dust, instruments, and all materials that may contact the samples. The best equipment to use for trace metal analyses is made of quartz, TFE (tetrafluoroethylene), polypropylene, or polyethylene. Stainless steel that is resistant to corrosion may be used if necessary. Corrosion-resistant stainless steel is not magnetic, and thus can be distinguished from other stainless steels with a magnet. Stainless steel scalpels have been found not to contaminate mussel samples (Stephenson et al., 1979). However, low concentrations of heavy metals in other biological tissues (e.g., fish muscle) may be contaminated significantly by any exposure to stainless steel. Quartz utensils are ideal but expensive. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for removing tissue for analysis. For bench liners and bottles, borosilicate glass would be preferred over plastic if trace organic analyses are to be performed on the same sample.

11.5.1.4 Resection should be conducted by or under the supervision of a competent biologist. Special care must be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The procedure previously outlined for the preparation of fillet samples should generally be followed. Unless specifically sought as a sample, the dark muscle tissue that may exist in the

vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass.

11.5.1.5 Prior to use, utensils and bottles should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. For quartz, TFE, or glass containers, use 1+1 $HNO_3$, 1+1 HCl, or aqua regia (3 parts conc. HCl + 1 part conc $HNO_3$) for soaking. For plastic material, use 1+1 $HNO_3$ or 1+1 HCl. Reliable soaking conditions are 24 h at 70°C (APHA, 1989; 1992). Do not use chromic acid for cleaning any materials. Acids used should be at least reagent grade. For metal parts, clean as stated for glass or plastic, except omit the acid soak step. If trace organic analyses are to be performed on the same samples, final rinsing with methylene chloride is acceptable.

11.5.1.6 Sample size requirements can vary with tissue type (e.g., liver or muscle) and detection limit requirements. In general, a minimum sample size of 6 g (wet weight) is required for the analysis of all priority pollutant metals. To allow for duplicates, spikes, and required reanalysis, a sample size of 50 g (wet weight) is recommended. Samples can be stored in glass, TFE, or high-strength polyethylene jars.

11.5.2 Processing

11.5.2.1 Samples should be frozen after resection and kept at -20°C. Although specific holding times have not been recommended by USEPA, a maximum holding time of 6 months (except for mercury samples, which should be held a maximum of 28 days) would be consistent with that for water samples.

11.5.2.2 When a sample is thawed, the associated liquid should be maintained as a part of the sample. This liquid will contain lipid material. To avoid loss of moisture from the sample, partially thawed samples should be homogenized. Homogenizers used to grind the tissue should have tantalum or titanium parts rather than stainless steel parts. Stainless steel blades used during homogenization have been found to be a source of nickel and chromium contamination. Some trace metal contamination during processing cannot be avoided and it is therefore necessary to determine and control the amount of contamination introduced during processing. Contamination can be monitored by introducing a dry ice blank into the blender and analyzing the chips.

11.5.2.3 To avoid trace metal contamination during processing the preferred method is to proceed to a chemical digestion process which minimizes or eliminates resection, homogenization, or grinding. Chemical digestion is best limited to specific organ tissues from large fish or to smaller sized whole fish.

11.6 Identification of Composite Whole Fish or Fillet Samples

11.6.1 Composite whole fish samples will be made up of three to ten fish with any deviation in number clearly identified. The limitation on the variance between individual fish in each composite will be as previously described. The length and weight of each fish must be recorded. The same field

information should be provided as described below for both fillet and/or whole body composite samples. The same handling precautions as described above should be followed for either organic or trace metal contaminants. Spines on whole fish should be sheared to minimize puncturing the sample packaging.

11.6.2 The following information should be included on the field/lab form for each sample collected:

11.6.2.1 Project Name

11.6.2.2 Station Code (if applicable)

11.6.2.3 Date

11.6.2.4 Collector's Name

11.6.2.5 Sampling location (river mile and/or other specific information relating to local landmarks)

11.6.2.6 Latitude and Longitude

11.6.2.7 Water body name

11.6.2.8 Sampling technique(s), i.e. 230 vac electrofishing apparatus, hoop nets, etc.

11.6.2.9 Fish species

11.6.2.10 Individual lengths and weights of fish in sample

11.6.2.11 Sample type (Whole or Fillet)

11.6.2.12 Individual fillet weights (whether left or right)

11.6.2.13 Comments or Unusual Conditions, i.e., tumors, sores, fin rot, blind, etc.

11.7 Chain-of-Custody Procedures (USEPA, 1990c; USEPA, 1991)
Also See Section 2, Quality Assurance and Quality Control.

11.7.1 All samples should be kept in a secure (locked) area to avoid legal complications in administrative proceedings. Transportation of the samples must be coordinated between the agency responsible for the field collection and the agency responsible for analytical work. When custody of the samples is transferred, the following checks should be implemented:

11.7.1.1 All transfers should be properly relinquished to ensure chain-of-custody. Transfers should be recorded on a form separate from the field data sheet. The chain-of-custody form should include the sample identification number(s). Custody tags must be used and numbered in sequence (if possible).

11.7.1.2 The field data sheet should stay with the sample until it is logged in by the analytical laboratory.

11.7.1.3 Samples can be shipped and chain-of-custody maintained as long as shipping containers are sealed with custody tape.

11.7.1.4 Samples should remain frozen until they are prepared for analysis. Shipping with dry ice is recommended.

11.7.1.5 The laboratory's receiving agent should initial the field data sheet and affix the date of sample receipt. Depending on administrative need, a copy of this form (with initials and date of sample receipt plainly visible) may be required by the lab agency's central office.

11.8 Conclusion

11.8.1 This protocol only addresses the steps to be considered in field sampling fish and sample preparation for human health fish consumption advisories and ecological risk assessment. Additional protocols must be followed to carry out the appropriate analytical chemistry and the risk assessment/management requirements leading to an action. These additional protocols were beyond the scope of this assignment.

11.9 Literature Cited

Cunningham, P.A., J.M. McCarthy and D. Zeitlin 1990. Results of the 1989 Census of State Fish/Shellfish Consumption Advisory Programs. Prepared for S.M. Kroner, Assessment and Watershed Protection Division, OWRS, USEPA, by Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC.

APHA. 1989. Standard methods for examination of Waste and Wastewater. 17TH Ed. American Public Health Association, Washington, DC.

APHA. 1992. Standard methods for examination of Waste and Wastewater. 18TH Ed. American Public Health Association, Washington, DC.

Hesse, John L. Michigan Department of Public Health, 1990. Summary and Analysis of Existing Sportfish Consumption Advisory Programs in the Great Lakes Basin. The Great Lakes Fish Consumption Advisory Task Force Co-Chaired by H.A. Anderson and L. Liebenstein, State of Wisconsin. Unpublished.

NOAA. 1989. A summary of data on tissue contamination from the first three years (1986-89) of the mussel watch project. Technical Memorandum, NOS, OMA49. Rockville, MD.

Puget Sound Estuary Program 1986. Recommended Protocols for Measuring Metals in Puget Sound Water, Sediment and Tissue Samples. Prepared by Tetra Tech, Inc., Bellevue, WA. *In:* Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound. USEPA, Region 10, Seattle, WA (Looseleaf).

Puget Sound Estuary Program 1989 (Revised). Recommended Guidelines for Measuring Organic Compounds in Puget Sound Sediment and Tissue Samples. Prepared by Tetra Tech, Inc., Bellevue, WA. *In*: Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound. USEPA, Region 10, Seattle, WA (Looseleaf).

Stephenson, M.D., M. Martin, S.E. Lange, A.R. Flegal and J.H. Martin 1979. California Mussel Watch 1977-78. Volume II: Trace metals concentrations in the California mussel, *Mytilus californianus*. SWRCB Water Quality Monitoring Report No. 79-22. Sacramento, CA.

USEPA, 1989. Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish: A Guidance Manual. EPA-503/8-89-002. Office of Marine and Estuarine Protection and Office of Water Regulations and Standards, Washington, DC.

USEPA, 1990a. Bioaccumulation of Selected Pollutants in Fish, A National Study Volume I and II. EPA-506/6-90/001. Office of Water Regulations and Standards (WH-552), Washington, DC.

USEPA, 1990b. Extraction and Analysis of Organics in Biological Tissue, Method OB 8/90, USEPA, Environmental Services Division, Region IV, Analytical Support Branch, Athens, GA.

USEPA, 1990c. Manual for the certification of laboratories analyzing drinking water. Criteria and procedures quality assurance. EPA/570/9-90/008. Prepared by the Laboratory Certification Program Revision Committee. Office of Water (WH-550D), Washington, DC.

USEPA, 1991. Manual for the evaluation of laboratories performing aquatic toxicity tests. EPA/600/4-90/031. Klemm, D.J., L.B. Lobring, and W.H. Horning, II. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.